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## REDUCED GLUTEAL EXPRESSION OF ADIPOGENIC AND LIPOGENIC GENES IN BLACK SOUTH AFRICAN WOMEN IS ASSOCIATED WITH OBESITY-RELATED INSULIN RESISTANCE

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### Abstract

**Context**—Black South African women are less insulin sensitive than their white counterparts, despite less central and greater peripheral fat deposition. We hypothesized that this paradox may be explained, in part, by differences in the adipogenic capacity of subcutaneous adipose tissue (SAT).

**Objective**—To measure adipogenic and lipogenic gene expression in abdominal and gluteal SAT depots, and determine their relationships with insulin sensitivity ( $S_I$ ) in South African women.

**Design**—Cross-sectional.

**Participants**—14 normal-weight (BMI <25 kg/m<sup>2</sup>) black, 13 normal-weight white, 14 obese (BMI >30 kg/m<sup>2</sup>) black and 13 obese white premenopausal South African women.

**Main outcomes**— $S_I$  (frequently sampled intravenous glucose tolerance test) in relation to expression of adipogenic and lipogenic genes in abdominal and gluteal SAT depots.

**Results**—With increasing BMI, black women had less visceral fat ( $P=0.03$ ) and more abdominal ( $P=0.017$ ) and gynoid ( $P=0.041$ ) SAT but had lower  $S_I$  ( $P<0.01$ ) than white women. The expression of adipogenic and lipogenic genes was proportionately lower with obesity in black, but not white women in the gluteal and deep SAT depots ( $P<0.05$  for ethnicity x BMI effect). In black women only, the expression of these genes correlated positively with  $S_I$  (all  $P<0.05$ ), independently of age and fat mass.

**Conclusions**—Obese black women have reduced SAT expression of adipogenic and lipogenic genes compared to white women, which associates with reduced  $S_I$ . These findings suggest that

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obesity in black women impairs SAT adipogenesis and storage, potentially leading to insulin resistance and increased risk of type 2 diabetes.

### Keywords

insulin sensitivity; ethnicity; adipogenesis; lipogenesis; adipose tissue distribution; hypertrophic obesity

## INTRODUCTION

Despite a high prevalence of insulin resistance, black South African women have less visceral adipose tissue (VAT) (1) and more peripheral (gluteal-femoral) subcutaneous adipose tissue (SAT) than their white counterparts (2). While increased VAT is considered a major determinant of insulin resistance (3), peripheral SAT deposition has been shown to be 'protective' (4), being inversely associated with fasting insulin levels in overweight and obese white premenopausal women (5). The mechanisms underlying this apparent paradox are not known.

The adipogenic capacity of SAT has been proposed as a potential mechanism underlying the link between adiposity and insulin resistance. Reduced adipogenic capacity of SAT is typically associated with increased adipose cell size (6), apoptosis, inflammation, reduced vascularization and insulin signaling within adipose tissue (7) and peripheral insulin resistance (8). As adipogenesis is controlled by a sequential activation of transcription factors, in particular, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ), which function with other adipogenic transcription factors, including sterol regulatory element-binding protein 1 (SREBP1), to regulate the expression of lipogenic genes such as lipoprotein lipase (LPL), fatty acid synthase (FASN), fatty acid binding protein 4 (FABP4) and adiponectin (9), we hypothesized that a reduction in these genes may be associated with insulin resistance in black South African women.

Therefore, we sought to measure expression of genes involved in adipogenesis and lipogenesis in abdominal and gluteal SAT depots and determine their relationships with insulin sensitivity in normal-weight and obese black and white South African women.

## METHODS AND PROCEDURES

The study population consisted of 14 normal-weight (BMI <25 kg/m<sup>2</sup>) black, 13 normal-weight white, 14 obese (BMI >30 kg/m<sup>2</sup>) black and 13 obese white pre-menopausal South African women, who were recruited by advertisement as previously described (1, 10). Briefly, inclusion criteria were: (i) age 18-45 years; (ii) no known diseases and not taking medication for any other metabolic diseases; (iii) not currently pregnant or lactating; and (iv) of South African ancestry. The study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town, and all subjects gave written informed consent.

The testing procedures, including the assessment of body composition, insulin sensitivity and adipose tissue biopsies, have been previously reported (1, 10). In brief, body fatness and gynoid fat mass (11), were assessed using dual-energy-X-ray absorptiometry (DXA, Discovery-W, Software version 4.40, Hologic Inc., Bedford, MA, USA), and abdominal VAT, deep SAT (DSAT) and superficial SAT (SSAT) areas was assessed by computerized tomography (CT, Toshiba X-press Helical Scanner, Japan). The insulin sensitivity index ( $S_I$ ) was quantified using Bergman's minimal model of glucose kinetics (12) from an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT).

Fat biopsies were obtained from the abdominal DSAT, SSAT and gluteal areas by mini-liposuction and used to measure adipocyte area and gene expression (10). Total RNA was isolated using the Qiagen RNeasy system (Qiagen Ltd, Crawley, UK). RT-PCR was performed in triplicate using a Roche LightCycler®480 (Roche Diagnostics Ltd, Burgess Hill, UK) with gene specific primers (Invitrogen Ltd, Paisley, UK) and fluorescent probes from either the Roche Universal Probe Library system or predesigned assays from Applied Biosystems (Warrington, UK) (Supplementary Table 1). Transcript levels are presented as the ratio of abundance of the gene of interest: mean of abundance of PPIA, 18S and RPLO.

Results are presented as means  $\pm$  standard error (SE). Differences in mRNA levels between ethnicity and BMI groups within each SAT depot were analyzed using two-way ANOVA with Bonferroni post-hoc analysis, with and without covarying for age and  $S_I$ . Partial correlations, adjusting for age and fat mass, were used to explore the associations between gene expression and measures of insulin sensitivity in both black and white women. The interaction between gene expression and ethnicity on insulin sensitivity was tested using multiple regression, including age and fat mass as covariates. Data were analyzed using STATISTICA version 10 (Statsoft Inc., Tulsa, OK).

## RESULTS

### Subject characteristics

The characteristics of the subjects have been described in detail previously (1, 10, 14). In brief, black and white women were well matched for % body fat and waist circumference, while obese black women had less VAT and more SSAT than white women, but similar DSAT. After adjusting for total fat mass, obese black women had greater gynoid fat mass (DXA-derived) than obese white women ( $P=0.041$ ), which correlated inversely with  $S_I$  in black ( $r=-0.44$ ,  $p=0.033$ ) but not white women ( $r=0.29$ ,  $p=0.22$ ). Fasting glucose levels were not different between ethnic groups, but black women had higher fasting insulin levels and a lower  $S_I$  than white women. Circulating adiponectin levels did not differ by ethnicity, nor did average abdominal and gluteal adipocyte size. There was no difference in the proportion of subjects with a family history of diabetes.

### Adipose tissue gene expression

Transcript levels of adipogenic and lipogenic genes in adipose tissue depots are presented in Figure 1. There were significant ethnicity  $\times$  BMI interactions for SREBP1 ( $P=0.041$ ) and FASN ( $P=0.017$ ) in the DSAT depot; and for PPAR $\gamma$  ( $P=0.004$ ), PEPCK ( $P=0.017$ ) and FABP ( $P=0.017$ ) in the gluteal depot, such that mRNA levels were significantly reduced with obesity in black, but not white women. These interactions were also significant after covarying for  $S_I$ . Adiponectin mRNA levels in both DSAT and gluteal depots trended to decrease to a greater extent with obesity in black compared to white women after covarying for  $S_I$  ( $P=0.054$ ,  $P=0.060$ , respectively). No ethnicity  $\times$  BMI interactions were observed in the SSAT depot.

Obesity also had effects independently of ethnicity. Within the gluteal depot only, mRNA levels of all adipogenic transcription factors were lower in obese compared to normal-weight women (all  $P<0.05$ ), whereas in all SAT depots, mRNA levels of all lipogenic genes were significantly lower in obese compared to normal weight women (all  $P<0.05$ ).

### Associations between adipose tissue gene expression and insulin sensitivity

In black but not white women,  $S_I$  correlated positively with the expression of genes involved in adipogenesis: CEBP $\alpha$  ( $r=0.47$ ,  $P=0.023$  and  $r=-0.04$ ,  $P=0.869$ ) and PPAR $\gamma$  ( $r=0.40$ ,  $P=0.057$  and  $r=-0.00$ ,  $P=0.992$ ), lipogenesis: FASN ( $r=0.52$ ,  $P=0.011$  and  $r=0.33$ ,  $P=0.135$ ),

LPL ( $r=0.50$ ,  $P=0.015$  and  $r=-0.15$ ,  $P=0.493$ ), FABP ( $r=0.46$ ,  $P=0.027$  and  $r=-0.42$ ,  $P=0.052$ ) and adiponectin ( $r=0.44$ ,  $P=0.034$  and  $r=-0.09$ ,  $P=0.707$ ) in the gluteal depot, and SREBP1 ( $r=0.431$ ,  $P=0.040$  and  $r=0.01$ ,  $P=0.953$ ) and FASN ( $r=0.519$ ,  $P=0.011$  and  $r=0.33$ ,  $P=0.135$ ) in the DSAT depot, respectively. In contrast, in white but not black women,  $S_I$  correlated negatively with FABP mRNA levels in the SSAT depot ( $r=-0.434$ ,  $P=0.044$  and  $r=0.20$ ,  $P=0.354$ ). These associations did not however differ by ethnicity ( $P>0.05$  for ethnicity  $\times$  mRNA).

## DISCUSSION

The novel findings of the study were that the expression of PPAR $\gamma$  and PPAR $\gamma$ -responsive genes were down-regulated to a greater extent with obesity in black compared to white women. Further, the expression of these genes, mainly in the gluteal and DSAT depots, was associated with insulin sensitivity in black, but not white women.

In support of previous studies (15), we showed that the expression of adipogenic and lipogenic genes was down-regulated with obesity in SAT of both black and white women. Decreased expression of these genes may represent an adaptive process limiting further accumulation of fat mass. However, our observation that the expression of PPAR $\gamma$  and PPAR $\gamma$ -responsive genes was down-regulated to a greater extent with obesity in black compared to white women, suggests an ethnic-specific adaptation. These findings corroborate those of Smith et al. (16) who showed lower expression of genes regulating adipogenesis and lipogenesis (PPAR $\gamma$ , lipin-1, AGPAT2, SCD1 and ATGL) in abdominal SAT of African American than Caucasian women who were similarly obese and insulin resistant. Reduced expression of adipogenic and lipogenic genes has also been reported in insulin resistant compared to insulin sensitive subjects (6, 17, 18). Accordingly, we found expression of the major adipogenic transcription factors (PPAR $\gamma$  and CEBP $\alpha$ ) and PPAR $\gamma$ -responsive genes (FASN, LPL, FABP and adiponectin) were correlated with insulin sensitivity in black, but not white women.

Notably, these gene associations with insulin sensitivity were only significant in the gluteal, and to a lesser extent, the DSAT depot. These findings are of particular relevance as we have previously shown that insulin sensitivity in this cohort of black women was most closely associated with CT-determined DSAT area, whereas in the white women, VAT area was the most significant correlate of insulin sensitivity (14). We now show for the first time that gynoid fat mass was negatively correlated with insulin sensitivity in black, but not white women, contrasting to the prevailing hypothesis that peripheral (gluteal-femoral) SAT deposition is 'protective' (as reviewed in Manolopoulos et al. (4)). However, to our knowledge, these studies have only been undertaken in Caucasian populations, and it is not yet known whether the associations are consistent in other ethnic groups. We propose that increased gluteal fat deposition in black South African women is associated with down-regulation of PPAR $\gamma$  and PPAR $\gamma$ -responsive genes, thereby reducing insulin sensitivity.

The mechanism whereby the expression of adipogenic and lipogenic genes are down-regulated is most likely mediated by higher levels of inflammation. In obese black women we have shown higher SAT expression of chemokines and cytokines than in white women, with highest expression in the gluteal depot (10). Cytokines, in particular, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) have been shown to inhibit adipogenesis by suppressing the induction of PPAR $\gamma$  and C/EBP $\alpha$  and maintaining the activation of the Wnt-signalling pathway (19), as well as inhibiting the expression of lipogenic genes including LPL and FABP (20).

This study has a few limitations. The study is limited by a small sample size, and larger studies are required to verify the findings in both black and white women and determine

whether the relationships are ethnic-specific. The cross-sectional nature limits inferences about causality. We did not measure protein levels, or have a direct measure of adipogenesis. Despite marked ethnic differences in the expression of PPAR $\gamma$  and PPAR $\gamma$ -responsive genes, we did not show ethnic differences in mean adipocyte size measured in frozen sections, perhaps due to limitations of the method, as the size distribution of the adipocytes may be more important than the mean cell size (18).

In conclusion, compared to white women, obese black women have impaired SAT expression of PPAR $\gamma$  and PPAR $\gamma$ -responsive genes, and this is particularly the case in the gluteal SAT. These changes in gene expression are associated with a reduction in insulin sensitivity. These findings add to the weight of evidence refuting the hypothesis that black women display ‘healthy obesity’ due to their greater peripheral fat distribution, but rather suggest that obesity in black South African women impairs gluteal SAT adipogenesis and storage, potentially leading to insulin resistance and an increased risk of type 2 diabetes. Prospective studies including a larger sample are now justified to extend these findings.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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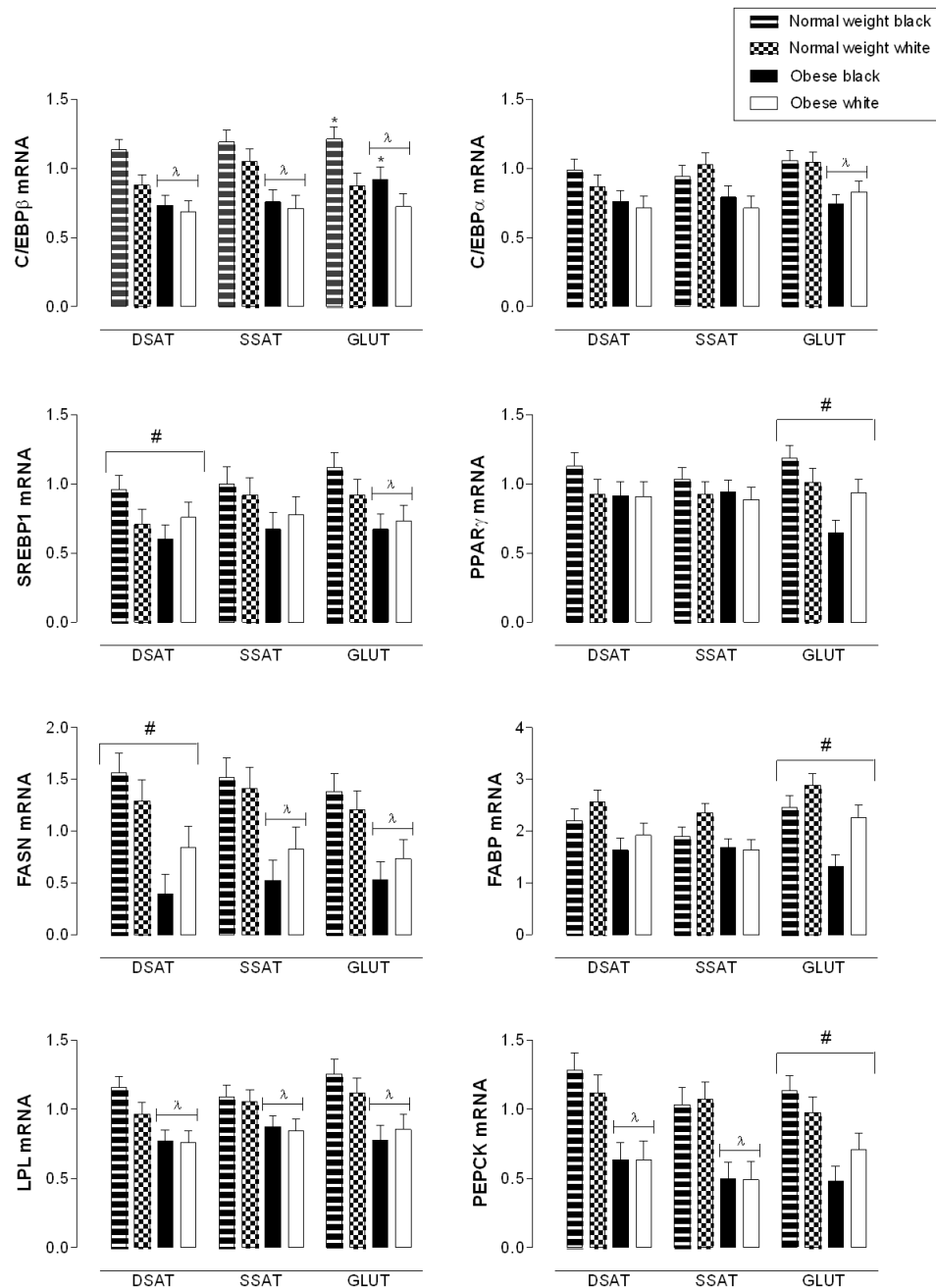
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**Figure 1. Expression of adipogenic and lipogenic genes in abdominal deep (DSAT) and superficial subcutaneous (SSAT) and gluteal (GLUT) adipose tissue depots in normal weight and obese black and white women.**

Bars represent means  $\pm$  SE. \* P<0.01, black vs. white;  $\lambda$  P<0.05, obese vs. normal weight; # P<0.05, ethnicity x BMI interaction effect (obese black women significantly different from all other groups). C/EBP $\beta$ , CCAAT/enhancer binding protein  $\beta$ ; C/EBP $\alpha$ , CCAAT/enhancer binding protein  $\alpha$ ; SREBP1, sterol regulatory element-binding protein 1; PPAR $\gamma$ , peroxisome proliferator activated receptor  $\gamma$ ; FASN, fatty acid synthase; FABP4, fatty acid binding protein 4; LPL, lipoprotein lipase; PEPCK, phosphoenolpyruvate carboxykinase.